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Full Length Research Paper

Characterization and determination of the factors affecting anti-listerial bacteriocins from *Lactobacillus plantarum* and *Pediococcus pentosaceus* isolated from dairy milk products

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Lactobacillus plantarum and Pediococcus pentosaceus **strains isolated from dairy milk products, produced bacteriocins that displayed a wide spectrum of antimicrobial activity against *Listeria ivanovii* and food spoilage microorganisms. The two bacteriocins were thermally stable over a wide temperature range up to 100°C for 15 min and retained their activity at pH 2.0 to 6.0. Full bacteriocins activity were stable after three months of storage at 4 and -20°C, for 75 min of exposure to UV light, bacteriocin produced by *P. pentosaceus* was completely destroyed, but the second *L. plantarum* remained stable after the same time of exposure; treatment with proteolytic enzymes resulted in a remarkable stability of activity. Results obtained showed an increase in bacteriocins activity of the both strains against *L. ivanovii* on increasing the concentration of NaCl and KCl up to 5%, in presence of spices 5%, stability to acetone and hexane indicated that both surfaces were rather hydrophilic and bipolar. Further studies are required regarding suitable bioprocessing strategies for an efficient bacteriocin production process.**

Key words: *Lactobacillus plantarum*, *Pediococcus pentosaceus*, bacteriocin activity, temperature, pH, enzymes, solvents, salts, spices.

INTRODUCTION

Lactic acid bacteria (LAB) are wide spread in nature and predominate in microflora of milk and its products; many species are involved in the daily manufacturing of dairy products.

The LAB used in commercial starter cultures possesses numerous metabolic characteristics such as acidification activity, proteolytic activity, synthesis of bacteriocin, resistance to bacteriophage and production of exopolysaccharide are strain dependent.

All of these important activities contribute to the flavour, texture, and frequently the nutritional attributes of the products. Research has been focused on the role of starter and its required properties for the dairy industries (Crow et al., 1993; El-Soda et al., 1995; Limsowtin et al., 1996; Mayra.a-Makinen and Bigret, 1998; De Vuyst and Degeest, 1999).

Bacteriocins are bactericidal peptides or proteins produced by bacteria that inhibit the growth of closely related bacterial species (Klaenhammer, 1993). Currently, interest in bacteriocins is intense due to their inhibitory activity against food spoilage and foodborne pathogenic bacteria such as Listeria monocytogenes (Yamazaki et al., 2003).

So far, only nisin produced by *Lactococcus lactis*, is a commercial product and an approved food additive in most major food producing countries. *Lactobacillus plantarum* produced serial bacteriocins that were active against the pathogens and spoiling microorganisms in foods like plantaricin35d,bacteriocinsST28MSandST26MS(Todorov

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and Dicks, 2005).

Another bacteriocin that attracts research interest and Will likely be the next to be used in the food industry is pediocin (Ray, 1992; Turcotte et al., 2004), which is an antilisterial bacteriocin (Guyonnet et al., 2000; Simon et al., 2002) produced by several Pediococcus strains.

However, strains of *Pediococcus acidilactici* and *Pediococcus pentosaceus* have been reported to produce bacteriocins (Gonzales and Kunka, 1987; Biswas et al., 1991; Kim et al., 1992; Elegado et al., 1997; Gurira and Buys, 2005).

Some of these are designated as pediocin AcH by *P. acidilactici* H, E, F, and M (Bhunia et al., 1987; Ray et al., 1989; Kim et al., 1992), pediocin PA-1 by *P. acidilactici* PAC 1.0 (Gonzales and Kunka, 1987), pediocin JD by *P. acidilactici* SJ-1 (Schved et al., 1993), pediocin 5 by *P. acidilactici* UL5 (Huang et al., 1996), pediocin A by *P. pentosaceus* FBB-61 (Etchells et al., 1964; Flemming et al., 1975), pediocin N5p by *P. pentosaceus* (Strasser de Saad et al., 1995), and pediocin ST18 by *P. pentosaceus* (Todorov and Dicks, 2005).

Pediococcus damnosus has also been reported to produce a pediocin, designated as pediocin PD-1 (Green et al., 1997). It is now known for most studied pediocins that they are plasmid encoded (Ray, 1995; Le Marrec et al., 2000) and posttranslationally modified hydrophobic molecules (Green et al., 1997; Yin et al., 2003; Wu et al., 2004), which also share a similar N-terminal sequence (Henderson et al., 1992; Wu et al., 2004).

Pediocins form a group of bacteriocins belonging to the class IIa of bacteriocins, characterized as “antilisterial” (Papagianni, 2003). They inhibit several gram-positive spoilage and pathogenic bacteria.

The spectra of antimicrobial activity of pediocins produced by strains of *P. acidilactici* and *P. pentosaceus* have been found to be similar and this has been attributed to the phylogenetically close relation of the producer organisms (Collins et al., 1991).

Pediocin PD-1 by *P. Damnosus*, however, has been found to be inactive against other pediococci, a characteristic that makes it different from the known pediocins produced by *P. acidilactici* and *P. pentosaceus* strains (Green et al., 1997).

In this study, for the first time we have explored the isolation, identification, characterisation, and determination the factors affecting the activity of a novel antimicrobial peptide produced by *L. plantarum* and *P. pentosaceus* strains isolated from the dairy milk products.

MATERIALS AND METHODS

Isolation and selection of LAB strains

LAB strains were isolated from dairy milk products. Samples were plated directly on MRS and M17 agar (Merck, Germany) at 30°C for 2 to 3 days (pH 6.5) under aerobiosis and anaerobiosis conditions. They were routinely propagated and stored at -20°C supplemented with glycerol (20% v/v). Working cultures were sub-cultured twice (1% inoculum, 24 h, 30°C) prior to use.

Identification of the LAB isolates

The pure isolate selected as a potential bacteriocin - producer was identified on the basis of its cultural, morphological, physiological and biochemical characteristics. Selected LAB isolates were characterized by Gram stain, absence of spores and catalase test. Gram +ve, catalase and spores negative strains were maintained frozen until needed for antimicrobial activity testing (Cintas et al., 1998). Confirmation of the identification was based on the use of API 50 kits.

Bacterial strains

The indicator strain *Listeria ivanovii* used in this work was provided by the Laboratory of Bacteriology, Microbiology Department at the Faculty of Sciences, Es-Senia, Oran University. For the antimicrobial assay, the pathogenic culture (*L. ivanovii*) was grown in the nutrient agar media (NA) at pH 7.4 ± 0.2.

Determination of *in vitro* antimicrobial activity

The inhibitory activity of the selected LAB isolates against *L. ivanovii* was assayed by the agar spot test described by Fleming et al. (1975). The LAB isolates were spotted onto the surface of MRSm agar (with 2% glucose for *L. plantarum* and 4% maltose for *P. pentosaceus*) plates and incubated at 30°C for 24 h to allow colonies to develop.

L. ivanovii was inoculated into 10 ml of soft MRS agar (0.9% agar) and poured over the plate on which the LAB isolates were grown. After incubation at 30°C for 24 to 48 h under aerobiosis conditions, the plates were examined for the presence of inhibition zones. Inhibition was considered positive when the width of the clear zone around the colonies of the LAB isolates was 0.5 mm or larger.

Preparation of cell free supernatant (CFS)

To prepare CFS stock of *L. plantarum* and *P. pentosaceus*, an overnight pre-culture of each isolated strain was inoculated as 2% into flasks containing MRSm broth (volume is variable according to the experiment need). Flasks were incubated at 30°C for 12 h till the stationary phase was reached. The CFSs were separated by centrifugation at 8000 xg at room temperature for 20 min to remove viable bacterial cells and any insoluble particulate matter. The resulting supernatants were filter sterilized using 0.2 µm pore bacterial filters (Renner GMBH D-67125/Germany). The filtered CFSs was stored in the refrigerator for a maximum period of 2 weeks and periodically tested for the bacteriocin titer before being renewed. The crude bacteriocin preparation was relatively stable when prepared in this manner, with an insignificant decline in titer over 2 weeks of storage.

Physical and biochemical characterization of bacteriocins

The isolated crude bacteriocins were characterized with respect to the effect of physical and biochemical parameters on bacteriocin activity. In all tests, the filter sterilized CFSs subjected to the different parameters were tested for their antimicrobial activity against *L. ivanovii* using the ODM method at 600 nm (Naclerio et al., 1993). Untreated bacteriocin-containing CFSs of each producer strain, inoculated with the same indicator strains served as controls.

Heat resistance

The thermal stability of crude bacteriocin preparations was asse-

ssed by exposing the CFSs to different temperatures (Mota et al., 2004) ranging from 0C to 121°C (0, 30, 40, 50, 60, 70, 80, 90, 100 and 121°C, and 15 Lbs) for 15 min before being tested for their antimicrobial activity.

pH sensitivity

The effect of pH on the activity of bacteriocins was tested by adjusting cell-free supernatants from 2 to 12 (at increment of one pH unit) with sterile 1 N NaOH or 1N HCl (Albano et al., 2007). After 1 h of incubation at room temperature (25°C), the samples were tested for antimicrobial activity by the ODM.

Stability during storage

The method devised by Ogunbanwo et al. (2003a) was used to study the stability of bacteriocin preparations during different storage conditions. The crude bacteriocin was stored at –20 and +4°C for different interval of time (30, 45, 60 and 90 days). Samples were taken from the stored material to determine the bacteriocin activity as previously mentioned. Bacteriocin-containing CFSs of each producer strain that were not subjected to storage, and inoculated with the same indicator strains served as controls.

Effect of UV light

The effect of UV light was studied according to the method of Ogunbanwo et al. (2003a). Sterile Petri dishes containing aliquots of 10 ml crude bacteriocin preparations were exposed to the UV irradiation (Philips bulb, wavelength 340 nm, 220 to 240 V, 50 Hz) situated at a distance of 30 cm (Wanda and Bonita, 1991). Times of exposure to UV light ranged from 15 to 75 min. After each time interval, bacteriocin activity was estimated to UV light by ODM as previously stated together with unexposed bacteriocin-containing CFSs that served as the experimental controls.

Sensitivity to proteolytic and other enzymes

Selected enzymes were tested on CFSs (Bizani and Brandelli, 2002). Proteolytic enzymes including trypsin, pepsin, and papain were dissolved in 40 mM Tris-HCl (pH 8.2), 0.002 M HCl (pH 7), and 0.05 M sodium phosphate (pH 7.0) respectively to a final concentration of 0.1 mg/ml. Other enzymes such as lipase and α-amylase were dissolved in 0.1 M potassium phosphate (pH 6.0), and 0.1 M potassium phosphate (pH 7.0) respectively to a final concentration of 0.1 mg/ml. Equal aliquots of both filter sterilized CFSs of each test strain and each enzyme solution were mixed, incubated at 30°C for each enzyme for 2 h and heated in boiling water for 5 min to inactivate the enzymes. These sample mixtures and the controls (CFSs without enzyme treatment) were inoculated with the indicator strains as previously mentioned and tested for antimicrobial activity by the ODM.

Effect of organic solvents on bacteriocin activity

The sensitivity of bacteriocins to organic solvents such as acetone, chloroform, alcohol 90%, and hexane was investigated (Todorov et al., 2006). Freeze dried bacteriocin preparations from each producer strain were dissolved individually in each organic solvent at a final concentration of 10 mg/ml. The samples were incubated at 30°C for 1 h, after which the solvents were removed by evaporation at room temperature. The dried residue from the organic phase was re-suspended in sterile MRS broth (Ten et al., 1994) at a final concentration of 10 mg/ml and assayed together with the untreated controls (CFSs without solvent treatment) for antimicrobial activity as previously mentioned.

Effect of inorganic salts on bacteriocin activity

Different concentrations of inorganic salts such as NaCl and KCl (0, 0.5, 1, 3 and 5 % w/v) were examined for their inhibitory effect on bacteriocin preparations (Karaoğlu et al., 2003). Equal aliquots of both filters sterilized CFSs of each test strain and each salt solution were mixed, inoculated with the indicator strains as previously mentioned. The antimicrobial activity of bacteriocins was determined for sample mixtures and controls using ODM.

Effect of spices used as food additives on bacteriocin activity

The possibility of influencing the effectiveness of bacteriocin activity by the use of different spices (black pepper, red pepper, and garlic) that are used as food additives, for the purpose of food preservation, was tested according to the method of Verluyten et al. (2004). All spices were obtained locally and used without prior sterilization. The concentrations of spices were chosen to ascertain the maximum effects of the spices possible. The spices separately weighed as 0.01% w/v, were allowed to dissolve in 10 ml of sterile warm distilled water and mixed overnight by stirring. Spice solutions were centrifuged and filter-sterilized. The CFSs were filter sterilized, to which different volumes of the spice extracts were added independently to give a final concentration of 0.5, 1 and 3% (v/v) then inoculated with the indicator strains. Controls were prepared by growing the indicator strains into the spice solutions without adding the bacteriocin-containing CFSs. The bacteriocin activity was determined by ODM at 600 nm as previously mentioned.

RESULTS

Two isolates were chosen for further experiments and characterization of their antimicrobial activity. Their selection was based on the display of high bacteriocin activity and their potential of inhibiting the growth of *L. ivanovii* using the agar spot method. The average diameter of the inhibition zone ranged from 1 to 14 mm in size (Figure 1).

The effects of heat and pH were determined. Bacteriocins of the two strains of LAB (Figures 2 and 3) were considered to be extremely heat stable as antibacterial activity was not altered by heat treatment after 15 min at 121°C.

The pH stability was studied in the range of pH 2 to 12. It was observed that these bactériocins were active at pH values from 2 to 6.0 but reduced at higher pH levels. pH adjustment to 6.5 was chosen to eliminate the possible effect of organic acids.

The influence of UV light on bacteriocin activity was studied on both organisms. It was observed that bacteriocin produced by *P. pentosaceus* was completely destroyed after 75 min exposure to UV light (Figure 4). However, the bacteriocin produced by *L. plantarum* remained stable after the same period of exposure time.

The bacteriocin from *L. plantarum* (Figure 5) was inactivated to the tested enzymes and displayed a higher growth reduction potential against *L. ivanovii* compared to

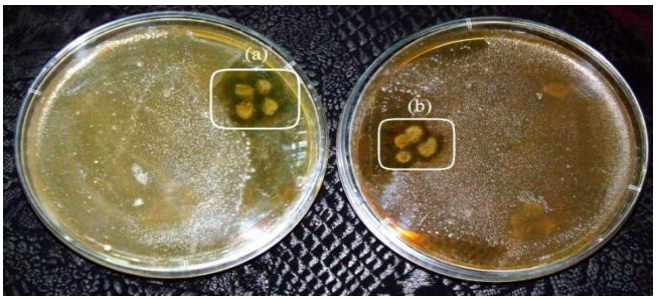

Figure 1. Anti-Listeria activity of bacteriocins from *L. pantarum* (a) and *P. pentosaceus* (b) determined by the agar spot on the MRSm agar plates at 30°C for 24 h.

that of *P. pentosaceus* which was sensitive to the rest of the selected enzymes (papain, pepsin, and lipase). However, since the activity of the filtrate was not completely inhibited, it is possible that the bacteriocin may also be bound to other molecules like a lipid or a carbohydrate moiety (Rakshita, 2011).

These data clearly show that the antimicrobial substance was of a proteinaceous nature. Storage of the active compounds at +4°C for three months and in a frozen state -20°C did not affect the antibacterial activity (Figure 6).

It was observed that the best organic solvents for activity of bacteriocin produced by *L. plantarum* hexane followed by acetone, where the activity was 50%, respectively. Concerning *P. pentosaceus*, the activity was stable when applying the tested organic solvents (hexane, acetone, and alcohol 90%) (Figure 7).

Different concentrations of NaCl and KCl (Figure 8) were selected to examine the effect of inorganic salts on the activity of the CFSs of the two LABs against the indicator strain used after 2 h of exposure. There was an increase in bacteriocin activity on the indicator strain with the increase in the concentrations of NaCl and KCl up to 5%.

The spices used in this experiment w/v (red pepper, black pepper, and garlic) in concentrations ranging from 0.5% affected the bacteriocins activity differently (Figure 9). The addition of 0.5% spices solution to both bacteriocins resulted in a significant activity against the indicator strains (up to 94% reduction of growth).

DISCUSSION

Bacteriocins could be interesting food conservatives on the industrial scale because of the thermostability of their relatively narrow spectrum of activity generally including Listeria and of their activity to weak concentrations on a broad range of value of pH. This study describes the partial characterization of a bacteriocins produced by *L. plantarum* and *P. pentosaceus* isolated from the dairy milk products. Choice was based on its broad antimicrobial spectrum against *L. ivanovii*. The ODM was preferred over the AWD assay in characterization experiments as aggregation, medium composition, and non- diffusible bacteriocins may influence the sensitivity of the AWD assay (Lewus et al., 1991).

In an attempt to characterize the physical and biochemical properties of the bacteriocins produced by the two strains presented in this study, different factors such as heat, pH, storage, UV light, enzymes, inorganic salts, organic solvents and spices were determined using *L. ivanovii* as indicator organism. Bacteriocins produced by *L. plantarum* and *P. pentosaceus* were heat stable for up to 15 min at 121°C. Storage for three months at -20 and 4°C did not affect bacteriocins activity. Full activity was retained even following incubation at 30°C for 2 h at pH values ranging from 2.0 to 6.0. These antimicrobial substances were sensitive to treatment with proteolytic enzymes, which was attributed to their proteinaceous nature. This is an interesting feature in view of its potential use as a food additive, in process like pasteurization, drying, tundalization, refrigeration and freezing.

Similar results reported by Suranjita et al. (2010) on the *L. lactis* W8 produced nisin concomitantly while fermenting milk to ‘‘dahi/curd’’, a traditional Indian fermented milk. Parada et al. (2007) indicated that some studies of characterization of bacteriocins show that these molecules can be active under certain ranges of temperature and pH. Sensibility to proteolytic enzymes evidences the proteinaceous characteristic of bacteriocins (De Martins et al., 2003). Complete inactivation or significant reduction in antimicrobial activity of the bacteriocins ST28MS and ST26MS produced by *L. plantarum* isolated from molasses was observed after treatment with proteinase K, pronase, pepsin and trypsin.

Ligocka et al. (2005) investigated that *Lactobacillus brevis* and *L. plantarum* isolated from compost green materials showed a high bactericidal activity (inhibition zone 1 to 4 mm) against *Listeria monocytogenes*, *Staphylococcus aureus* and *E*scherichia *coli*; but did not inhibit the growth of *Listeria innocua* strain. However, compared with the data given by Green et al. (1997) for pediocin PD-1 by *Pediococcus damnosus*, pediocin SA-1 appeared to be significantly more effective against *Listeria* spp. Purified pediocin SA-1 is heat stable for up to 60 min at 121°C. Storage for 4 weeks at -80, -20, +4 and 30°C did not affect bacteriocin activity. Full activity was retained even following incubation at 30°C for 1-week at pH values ranging from 3.0 to 12.0. No antimicrobial activity was detected after 30 min of incubation in the buffers of pH 2.0, 13.0 and 14.0. Purified pediocin SA-1 is resistant to treatment with trypsin, a-chymotrypsin, pepsin and papain, but not to proteinase K. (Anastasiadou et al., 2008).

The results reported by Diop et al. (2008) on bacteriocins produced by two strains of *L. lactis* subsp. *lactis* CWBI-B 1410 and CWBI-B1426 that were isolated from the fermented cereal and the seafood samples, indicated

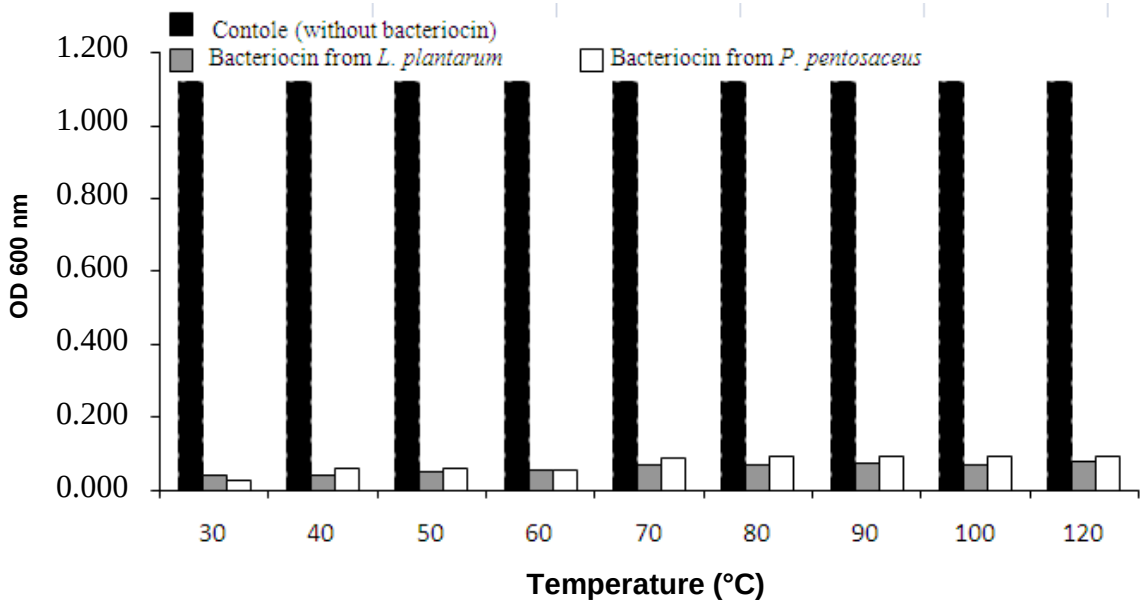

Figure 2. Effect of the heat treatment on antimicrobial activity of bacteriocins produced by *L. plantarum* and *P. pentosaceus.*

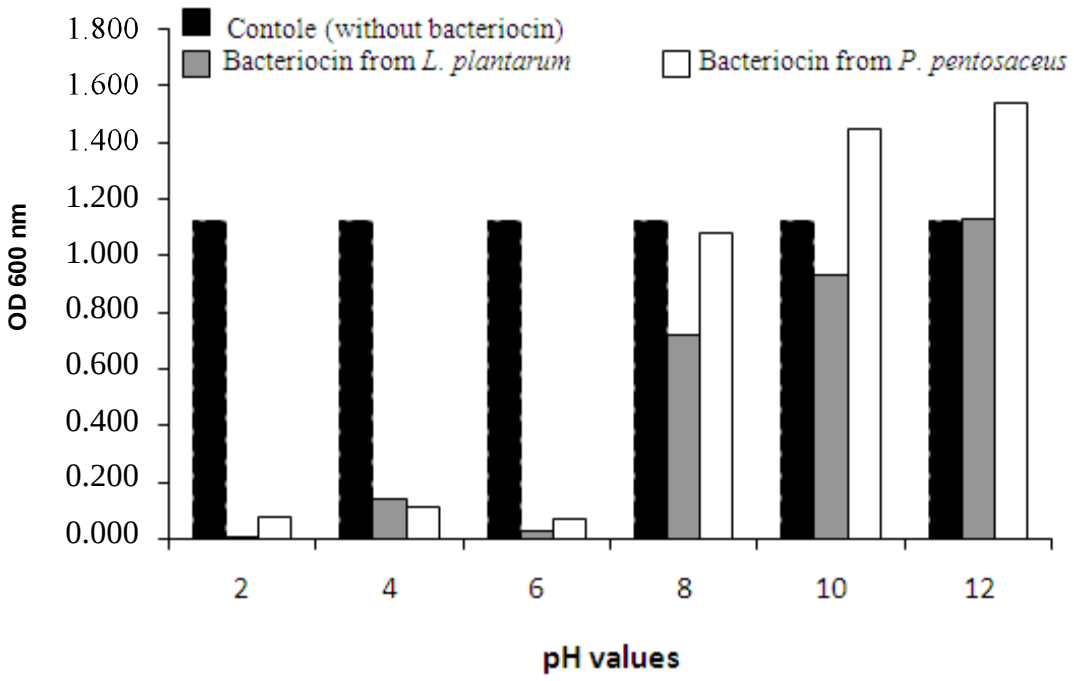

Figure 3. Effect of pH on the stability of inhibitors produced by *L. plantarum* and *P. pentosaceus*.

that they were digested by proteases and showed stability at 100°C for 30 min. Bromberg et al. (2005) showed that the bacteriocin-producing *L. lactis* subsp. *cremoris* CTC 204 was heat stable even at autoclaving temperature (121°C for 10 min) and was produced during refrigerated storage. Vinod et al. (2006) described that the bacteriocin produced by Lactobacillus CA44 was stable at up 100°C for 60 minutes but its activity declined compared to that at 68°C and was completely lost at 121°C for 15 min. Contradictory results were obtained by Valdes and Scherer (1994), where they found that the bacteriocin linocin M18 produced by Brevibacterium linens was heat sensitive. Joshil et al. (2006) reported that bacteriocin from *L. plantarum* CA44 (isolated from carrot fermentation) was

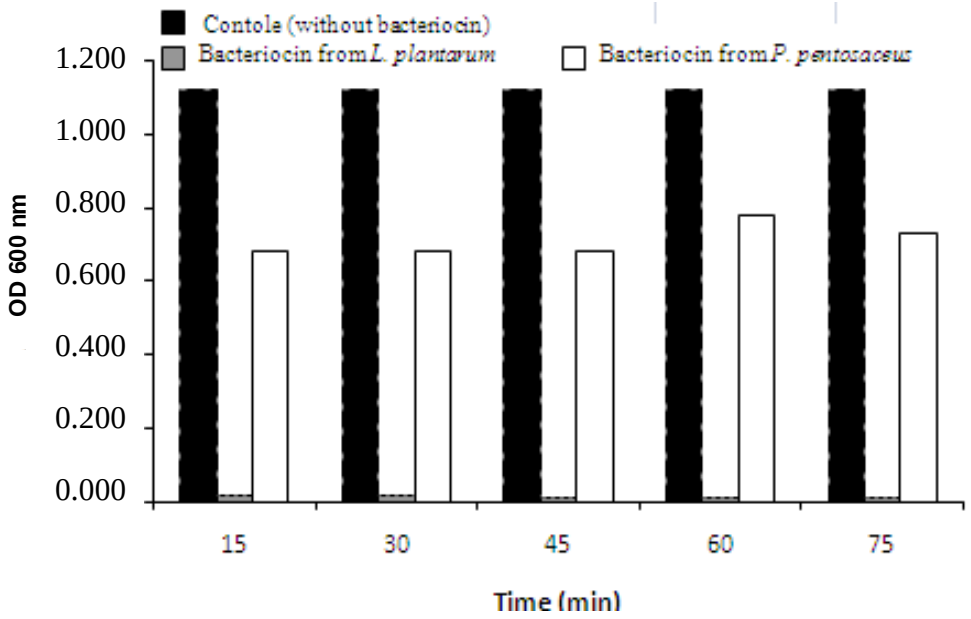

Figure 4. Effect of exposure to UV light on antimicrobial activity of bacteriocins produced by *L. plantarum* and *P. pentosaceus*.

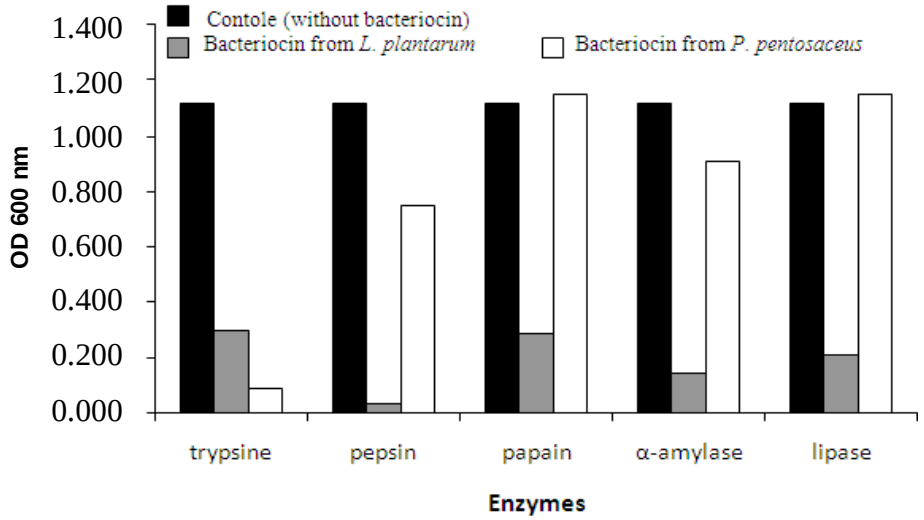

Figure 5. Study the effect of the proteolytic and non proteolytic enzymes (0.1 mg/ml) on the bacteriocins activity of the both strains *L. plantarum* and *P. pentosaceus* against *L. ivanovii* using the ODM method at 600 nm.

activated against *E. coli*, *S. aureus* and *Bacillus cereus*. It was stable up to 100°C but its activity declined at 68°C and was lost at 121°C. The maximum antimicrobial activity was retained with pH value of 4 to 5, but was adversely affected by the addition of papain.

Mkrtchyana et al. (2009) described that *Lactobacillus acidophilus* n.v. Er 317/402 strain Narine produces a small bacteriocin with a molecular weight of 1.1 kDa, designated acidocin LCHV. It was extremely heat stable (90 min at 130°C), was active over a wide pH range, and was found to be sensitive to proteolytic enzymes (trypsin, pepsin and proteinase K).

According to Navarro et al. (1999), *L. plantarum* J-51 bacteriocin remained stable at storage temperatures, such as 4, –20°C and room temperature, and under strong heating conditions (100°C for 60 min). Nevertheless, thermostability was lower after a previous storage at –20°C.

The influence of UV light on bacteriocin activity was studied on both organisms. It was observed that bacteriocin produced by *P. pentosaceus* was completely destroyed

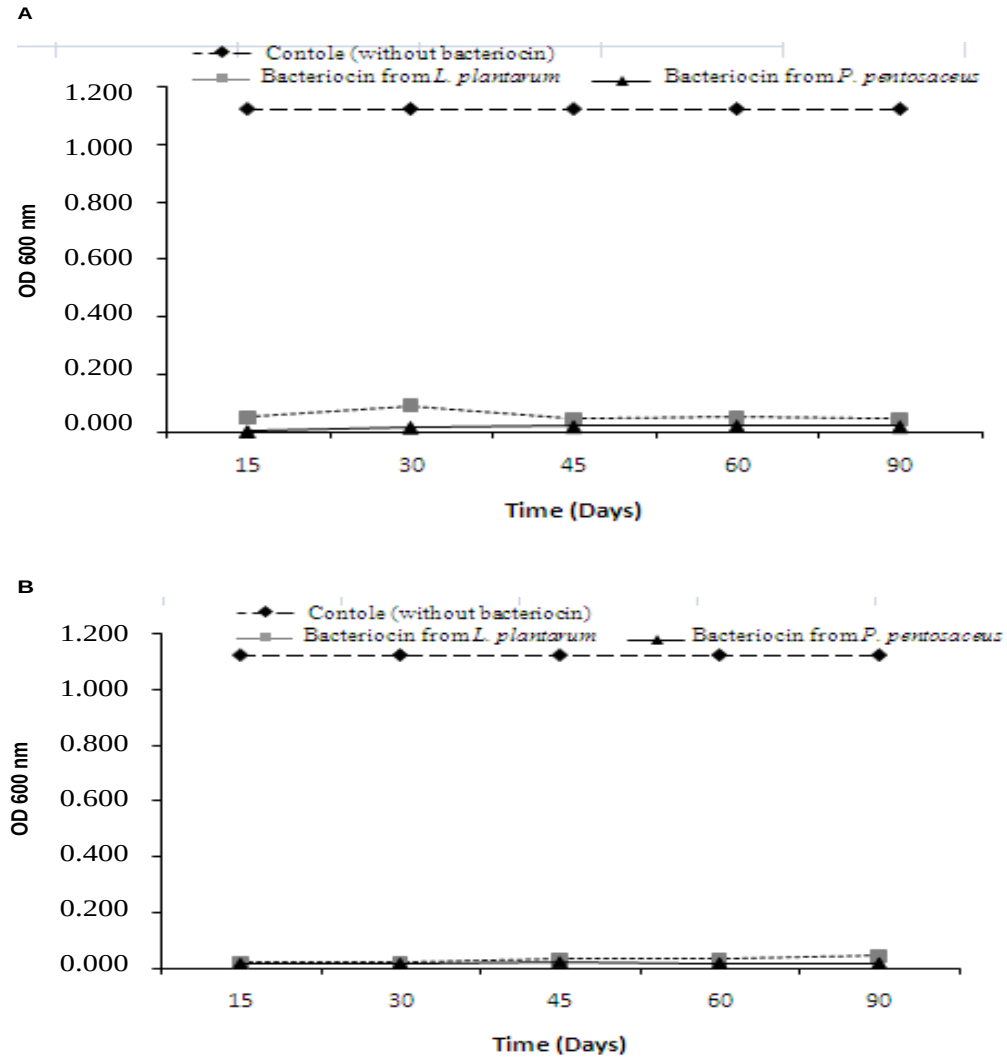

Figure 6. Effect of long time storage at +4°C (a) and -20°C (b) on the activity of bacteriocins produced by *L. plantarum* and *P. pentosaceus*.

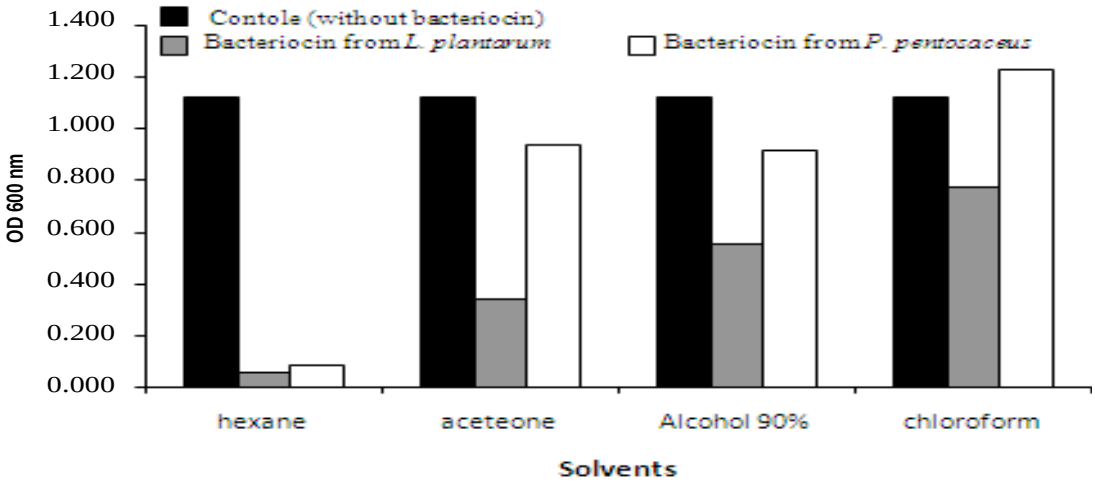

Figure 7. Sensibility of the bacteriocins of *L. plantarum* and *P. pentosaceus* strains to different solvents using *L. ivanovii* as indicator strain, using the ODM method at 600 nm.

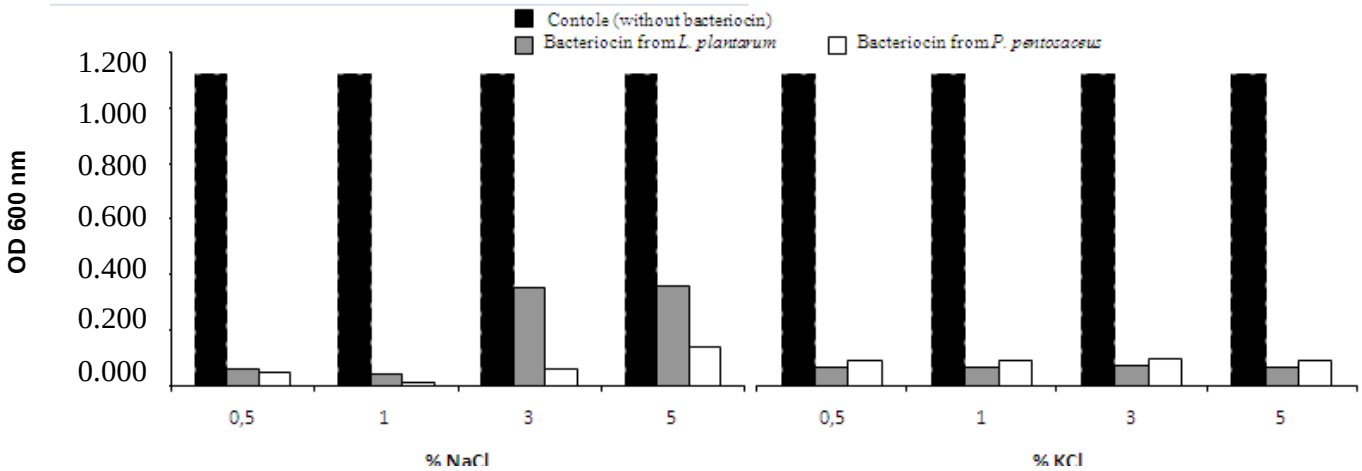

Figure 8. Study the effect of salts (NaCl and KCl) on antimicrobial activity of bacteriocins produced by *L. plantarum* and *P. pentosaceus*.

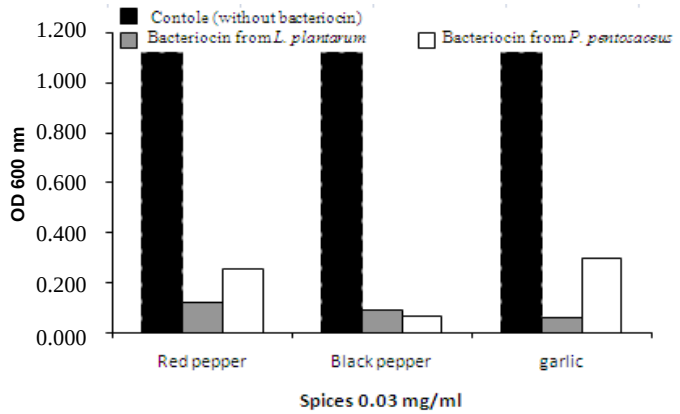

Figure 9. Study the effect of spices on antimicrobial activity of bacteriocins produced by *L. plantarum* and *P. pentosaceus.*

after 75 min exposure to UV light. However, the bacteriocin produced by *L. plantarum* remained stable after the same period of exposure time. Results from UV light were not surprising and confirmed the protein status of bacteriocins. This might be due to the effect of ultraviolet light on the protein nature causing modification and/or change in the ring structure of peptides, or affecting the protein function. The results are consistent with those described for bacteriocins produced by *L. plantarum* F1 and L. brevis OG1 after exposure to UV light from 0 to 5 min Ogunbanwo et al. (2003a)

The effect of some organic solvents on bacteriocin activity of the two LAB under test was also examined. Hexane, acetone, and alcohol 90% did not affect the activity of bacteriocin from P. pentosaceus. On the other hand, alcohol 90% and chloroform applied completely destroyed the bacteriocin produced from L. plantarum. These results suggest that acetone and hexane could be used in the extraction procedure to remove the bacteriocin from the aqueous phase and recover from the organic phase. Another explanation could prove the presence of lipid moiety in bacteriocin.

Some of results are in accordance to these stated for *P. pentosaceus*. Zeliha and Metin (2001) showed that the buchnericin LB produced by *Lactobacillus buchneri* LB completely lost its biological activity after treatment with organic solvents as formaldehyde, chloroform, acetone, 2-propanol, ethyl alcohol, hexane, iso-butanol and ethyl ether at a concentration of 10 mg/ml. Some of these results are similar to our results obtained for *L. plantarum*.

In order to show the effect of the addition of different ingredients on the bacteriocin efficacy, NaCl and KCl were used. There was an increase in bacteriocin activity of the both strains against L. ivanovii on increasing the concentration of NaCl and KCl up to 5%. These data suggest that the inorganic salts have a synergistic effect on bacteriocin efficacy when added with specific concentrations in foods.

Gänzle et al. (1999) reported that a synergistic effect has already been shown for curvacin A and sodium chloride, rendering the gram-negative pathogens *E. coli* and *Salmonella enterica* susceptible, while at low pH these pathogens also show increased sensitivity to the bacteriocins tested. Hagas et al. (2002) indicated that some common ingredients have a synergistic effect on bacteriocin efficacy or production, while some ingredients can reduce antimicrobial activity.

All of the spices tested in this study in concentrations of 5% did not affect bacteriocins activity differently. The addition of 5% solution spices to both bacteriocins resulted in a significant activity against the indicator strain (up to 94% reduction of growth). This indicates that small amounts of additives should be used in some foods to promote the activity of the bacteriocins applied, especially when more than one spice is used.

Extensive studies have been performed to determine the inhibitory properties of garlic, and many food-borne pathogens, both gram-positive and gram-negative bacte-

ria, have been shown to be inhibited by it (Kumar and Berwal, 1998; Ross et al., 2001; Leuschner and Zamparini, 2002). Singh et al. (2001) indicated that bacteriocins or bacteriocinogenic starter cultures can be applied in various food products and may provide a synergistic effect using nisin and garlic extract. In both, such a positive interaction between nisin and garlic extract has been shown towards strains of *L. monocytogenes*.

Ettayebi et al. (2000) and Singh et al. (2001) described that the combination of spices together with bacteriocins that, albeit in a lower amount, can be produced *in situ* by the bacteriocinogenic starter culture may lead to a synergistic effect, rendering pathogens susceptible to *L. monocytogenes.* Such a synergistic inhibition has been shown between nisin on the one hand and garlic extract or thymol on the other.

Chumchalova´ et al. (2004) reported that the partial purification and characterization of heat-stable acidocin CH5, a bacteriocin produced by *L. acidophilus* CH5, showed that the active compound caused fast decrease in viable cell count of the indicator strain *Lactobacillus* delbrueckii subsp. *lactis* LTI30 which was in accordance to results obtained in this study.

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